DOI:
10.1016/j.thromres.2016.04.002

Document Version
Peer reviewed version

Clinical and laboratory predictors of deep vein thrombosis after acute stroke

Ibrahim O. Balogun, Lara N. Roberts, Raj Patel, Rohan Pathansali, Lalit Kalra, Roopen Arya

PII: S0049-3848(16)30290-0
DOI: doi: 10.1016/j.thromres.2016.04.002
Reference: TR 6306

To appear in: *Thrombosis Research*

Received date: 27 January 2016
Revised date: 10 March 2016
Accepted date: 1 April 2016

Please cite this article as: Balogun Ibrahim O., Roberts Lara N., Patel Raj, Pathansali Rohan, Kalra Lalit, Arya Roopen, Clinical and laboratory predictors of deep vein thrombosis after acute stroke, *Thrombosis Research* (2016), doi: 10.1016/j.thromres.2016.04.002

Clinical and laboratory predictors of deep vein thrombosis after acute stroke

Ibrahim.O. Balogun[*†], Lara .N. Roberts†, Raj Patel†, Rohan Pathansali[**], Lalit Kalra[¶], Roopen Arya[†]

[†]King's Thrombosis Centre, Department of Haematological Medicine, King's College Hospital NHS Foundation Trust, London, UK

[**] Department of Stroke medicine, King's College Hospital NHS Foundation Trust, London, UK

[¶]Clinical Neuroscience Department, Academic Neuroscience Centre, King's College London, UK

[*]Department of Stroke medicine, East Kent Hospitals University NHS Foundation Trust, East Kent, UK

Background

The clinical utility of pretest probability models in the diagnosis of VTE has been well validated (1). However, research into VTE predictability models is inadequate (2), especially in the aftermath of acute stroke, when development of clot in leg veins can be as early as 48 hours and early in the second week (3, 4). The impact of improved and organised specialist stroke intervention, including thrombolytic therapy on clinical outcome is well known (5-7). However, the effects of this specialist input, thrombolytic therapy in ischaemic stroke, and thromboprophylaxis on incidence of VTE in the era of hyperacute stroke care is yet unknown. In previous studies, the incidence of symptomatic VTE is 1-20% (8, 9), with subclinical incidence of 8-75% (10-12). There remains lack of clarity in International guidelines on timing of thromboprophylaxis (13-16). Thus a VTE prediction model to identify those at high risk who will benefit from early thromboprophylaxis is desirable. We postulate that in the aftermath of acute stroke, and independent of the clinical intervention approach to the overall stroke care, markers of haemostatic activation due to endothelial perturbation and/or stroke severity may predict those at risk of developing DVT. We investigate the role of laboratory factors (thrombin generation, D-dimer, fibrinogen) alongside clinical factors (National Institute of Health Stroke Scale and Barthel Index)

in improving pretest probability. NIHSS is a measure of stroke severity and Barthel index is a measure of functional abilities. Thrombin generation is a novel global coagulation assay which has been used in non stroke patients as a research tool to risk stratify for recurrent VTE (17, 18), and in the diagnosis of first DVT (19-21).

Methods

Study objectives

1. To predict DVT after acute stroke using markers of haemostatic activation and stroke severity scores.
2. To define incidence of DVT in the era of hyperacute stroke care.
3. To design a pretest probability model to identify patients at high risk of DVT

Study design

Participants were prospectively recruited from consecutive in-patient stroke admissions at King's College Hospital NHS Foundation Trust, between June 2009 and August 2011. Participants with an objective diagnosis of stroke were invited to take part within 48 hours of admission. Patients with radiological diagnosis of stroke after 48 hours or those on end of life care within 48 hours of admission and life expectancy less than 7 days were excluded. Other reasons for exclusion were early repatriation to local hospitals, refusal to continue with study after screening, previous DVT/PE, previous diagnosis of cancer, prior anticoagulant therapy, and previous lower limb amputation. All participants had baseline NIHSS, BI recorded. The height (m) and weight (kg) of all participants were measured to allow calculation of body mass index (BMI). Past medical history, including past cardiovascular, cerebrovascular history, and medication history were recorded. All patients were screened for DVT in week 2 using compression Doppler ultrasound scan, as described below. If there was clinical suspicion of PE participants were investigated with CT pulmonary angiogram. All patients with diagnosis of ischaemic stroke received Aspirin 75mg once daily and Dipyridamole MR 200mg twice daily within 24 hours of admission. And considering there was no concensus on routine thromboprophylaxis. This was commenced after compression ultrasound scan in week 2 if no DVT was found. Otherwise patients with objective evidence of DVT were treated with bridging therapy of clexane and warfarin.

The study was approved by York Research Ethics Committee (REC reference number: 09/H1311/12), and the local Research and Development department at King's College Hospital, London. All patients gave informed written consent prior to data and sample collection.

Sample collection and plasma preparation

Upon enrolment in the study, venous blood was collected from the antecubital vein, via Butterfly-21 needle (Hospira Inc, IL, USA), with minimal venous stasis using a 10 ml syringe. Blood was immediately dispensed into 0.109M sodium citrate plastic (BD vacutainer, Plymouth, UK, nine parts blood to one part sodium citrate) following an initial 10 ml blood drawn which was subsequently added 0.369M ethylenediaminetetraacetic acid (EDTA) BD vacutainer (BD Diagnostics, Plymouth, UK). The same blood sample collection technique was carried out between days 7-10.

Platelet poor plasma (PPP) for thrombin generation was prepared by centrifugation of blood sample in the sodium citrate plastic vacutainer using Hettich Rotina 420R centrifuge (Hettich, Tuttlingen, Germany) at 4750*g* for 10 minutes, at room temperature. After the initial centrifugation, the top three-quarters of supernatant was decanted into a polypropylene tube and then centrifuged a second time at 4750g for 10 minutes again at room temperature. The top three-quarter of the supernatant was transferred into a plastic tube, and stored at -40^{o}C. Samples were processed and frozen within 60 minutes of collection.

Plasma for D-dimer and Clauss fibrinogen was prepared by single centrifugation at 3040*g* for 7 minutes and tested within 4 hours.

Thrombin generation was undertaken within 12 weeks of sample collection, following further centrifugation after plasma thawing at 4750g for 10 minutes. Intra-assay and inter-assay variability was < 5% and <12% respectively for all thrombin generation parameters.

Laboratory assays

Thrombin generation

Thrombin generation was measured in platelet poor plasma (PPP) with the Thrombinoscope^{TM} assay (Thrombinoscope BV, Maastrichts, Netherlands) as previously described (22, 23). Final tissue factor concentration was 5pM with 4µM of phospholipids. Parameters measured were lag time (minutes), time to peak (ttP, minutes), peak thrombin generation (nM) and endogenous thrombin potential (ETP), which is the area under the curve (nM.min).

Standard coagulation assay

D-dimer was measured by a latex photometric immuno-assay and Clauss fibrinogen using Diagnostica Stago reagents (Asnieres, France) on the automated STA® Evolution analyser(Diagnostica Stago) as described previously (24).

Duplex compression ultrasound scan

All participants had bilateral whole leg duplex compression ultrasound scan performed in week 2 (7-10 days), by a trained Vascular Scientist. The ultrasound devices used were Siemens Acuson Sequoia 512 (Erlangen, Germany) and Philips Healthcare iU22 (Eindhoven, The Netherlands) with a linear 3-6 MHz transducer. Lack of compressibility of a deep venous segment indicated the presence of DVT. Participants with diagnosis of DVT received therapeutic low molecular weight heparin or unfractionated heparin infusion in the presence of a contraindication to the former.

Statistical analysis

Continuous variables were given mean and standard deviation, or median and interquartile ranges for normal and non-normal data respectively. Categorical variables are presented as number and percentage. Comparisons were made between groups (with/without DVT) using the unpaired t-test for normally distributed continuous variables. Unpaired t-test was also used to find the mean difference between the 2-point coagulation assays. The Mann-Whitney test was used for non-normally distributed continuous variables. Fisher's exact test was used to compare categorical variables between those with and without DVT. Where applicable, odd

ratios were obtained from logistic regression in an adjusted and unadjusted analysis as a measure of association between variables tested and DVT. Subsequently, the joint effect of the variables upon development of DVT was evaluated using multivariable logistic regression analysis. To limit the number of variables in this analysis, only factors with a $p \leq 0.2$ from univariable analysis were included in this analysis. A backward selection procedure was used to retain only the statistically significant variable. Adjustments were made for the effect of cofounder factors (age, body mass index, Barthel index, NIHSS) on the statistically significant variables. Distribution of D-dimer was positively skewed and multimodal and as such underwent logarithm transformation to facilitate normal distribution and dichotomisation. We adjusted for the missing data from the observed data, by replacing missing values with predicted scores from the regression equation. This was done by single imputation. Further analysis was performed with receiver operating characteristic (ROC) curves, to determine the area under the ROC curve (AUC), which was used to identify the optimum cut-point. The optimum cut-point was the point which maximised the combination of sensitivity and specificity. Statistical significance was given a p-value of <0.05. All statistical analysis were performed using Stata version 12 software (Stata Corp LP, Texas, USA).

Results

Participant demographics and risk factors

Five hundred participants were screened, with exclusions as shown in the Study algorithm in figure 1.

188 participants gave consent and were recruited into the study. From this number, 96 participants withdrew due to early repatriation to local hospitals, medical complications such as severe septicaemia, bleeding diastheses, renal failure requiring frequent dialysis, upper gastrointestinal bleeding requiring intensive therapy unit admissions, New York Heart Association class IV heart failure and acute severe myocardial infarction. These participants were moribund and were as a result unfit for further investigations including Doppler ultrasound scan. The remaining participants (n=92) had complete investigations. Of these numbers, there were 9

haemorrhagic, and 83 ischaemic strokes. 18/92 participants had asymptomatic DVT diagnosed in the second week, of which 3 were in the haemorrhagic and 15 in ischaemic stroke. There were in total 4 proximal and 14 distal DVT. Of the patients with DVT, 6 had received intravenous recombinant tissue plasminogen activator (r-tPA) for ischaemic stroke.

Table 1, showed demographic details in those with and without DVT. There were no significant differences in age and gender distribution. With regard to the risk factors, diabetes was over represented in DVT patients and hypertension in those without DVT. BMI in those with DVT was higher than those without (28.6±4.6 versus 26.1±4.9; p=0.05). No other risk factors for stroke were associated with DVT. Of the total patients investigated (n=92), 29 patients were treated with intravenous r-tPA. Of these 21% (6/29) went on to develop DVT, constituting 40% (6/15) of all DVT in the ischaemic stroke subgroup.

Table 1. Demographic details and risk factors for stroke.

Category .	No DVT n=74	DVT n=18	*p*-value
Age,mean (SD)	68.9 ± 14.8	69.7 ± 13.4	0.83
Male, n (%)	35 (47)	9(50)	0.84
Ethnicity			
African-Caribbean, n (%)	29 (39)	6 (33)	0.18
White, n (%)	45 (61)	12 (67)	0.23
Risk factors			
Diabetes Mellitus, n(%)	14(19)	8(44)	**0.02**
Hypertension, n (%)	44(60)	6(33)	**0.03**
Smoking, n (%)	11(15)	4(22)	0.20
Atrial fibrillation, n (%)	13(18)	4(22)	0.22
Dyslipidaemia, n (%)	24(32)	3(17)	0.10
IHD, n (%)	6(8)	3(17)	0.19
Peripheral vascular disease, n (%)	4(5)	1(6)	0.42
BMI- mean (SD)	26.1± 4.9	28.6±4.6	**0.05**
Stroke severity			
NIHSS- mean (SD)	12.1± 7.1	15.6±7.7	0.07
NIHSS <7, n (%)	24(32)	4 (22)	-
NIHSS 8-14, n (%)	15 (19)	3 (17)	-
NIHSS > 14, n (%)	35 (47)	11 (61)	-
Motor limb weakness			
Paresis, n (%)	53(72)	17(94)	**0.03**
Functional rating			
Barthel index- mean(SD)	9.4±6.1	6.1±6.5	**0.05**
Barthel index < 9, n (%)	43(58)	13 (72)	-
Barthel index > 9, n (%)	31 (42)	5 (28)	-
Prestroke medications			
Antiplatelets, n (%)	8(11)	2(11)	0.32
Statins, n (%)	13 (18)	3(17)	0.27
Antihypertensive, n (%)	12(16)	4(22)	0.24
VTE prophylaxis			
GCS, n (%)	2 (2.7)	1(5.6)	-
IPC, n (%)	-	-	-
Enoxaparin, n (%)	1(1.4)	-	-
Unfractionated heparin, (%)	-	-	-
Time to acquisition of tested variables (All participants)			
NIHSS, median (IQR), min	28 (17,42)		
Barthel index, median (IQR), min	47 (17,6)		
NCCT, median (IQR), hours	3.8 (0.5, 23)		
Coagulation markers, median (IQR) hours	17 (10, 33)		
DtN time (rtPA), median (IQR), hours	47 (34, 84)		
CUSS, median (IQR), days	8.9 (7.2, 10.8)		

IHD-Ischaemic heart disease; NIHSS-national institute of health stroke scale; BMI-body mass index; DVT- deep vein thrombosis. CUSS- compression ultrasound scan

Relationship between clinical factors and DVT

Participants with DVT had worse stroke severity as indicated by higher NIHSS (15.6±7.7 versus 12.1±7.1; p=0.07), and when compared to the non DVT group, more patients in the DVT group had NIHSS greater than 14 (61% versus 47%), as shown in Table 1. There was significantly more motor limb weakness in the DVT group (94% versus 72%; p=0.03). Patients in the DVT group were functionally less able with lower BI group (6.1±6.5 versus 9.4±6.1; p=0.05), BMI in those with DVT was higher than those without (28.6±4.6 versus 26.1±4.9; p=0.05). There were 18 DVTs identified, out of which 6 were proximal, and 12 distal. In proximal DVT, 1 participant had bilateral DVT.

Relationship between thrombin generation and DVT

Table 2, shows thrombin generation results in patients with DVT versus those without DVT. Thrombin generation parameters did not differ between the groups at baseline and in the second week.

Relationship between D-dimer, fibrinogen and DVT

At both baseline, and week 2, patients with DVT had significantly higher D-dimer levels, as shown in Table 2. In the DVT group median D-dimer was significantly higher at baseline (2280ng/mL Vs 1035ng/mL, p=0.001) and in the second week (2240 ng/mL Vs 970 ng/mL; p<0.001).

A graphical illustration of the difference in D-dimer values between the two groups is shown in. Box plot in figure 2a showed the association between baseline D-dimer (time 1) and DVT, while box plot in figure 2b, showed the association between week 2, (time 2) D-dimer and DVT. There were no difference in fibrinogen levels between the two groups.

Table 2. Laboratory predictors of deep vein thrombosis

Test	No DVT (n=74)	DVT (n=18)	p-value
Thrombin generation assays			
Lag time 1, min- median (IQR)	3.2 (2.7, 4.0)	3.2 (2.8, 3.6)	0.96
Lag time 2, min- median (IQR)	3.4 (2.9, 4.2)	3.3 (3.0, 4.0)	0.65
Change in Lag time, min-mean (SD)	0.4 (1.3)	0.0 (1.2)	0.32
Baseline ETP, nmol/min-mean (SD)	1730 ± 348	1687 ± 355	0.66
Week 2 ETP, nmol/min- mean (SD)	1697 ± 375	1671 ± 234	0.93
Change in ETP, nmol/min-mean (SD)	-48 (416)	-58 (252)	0.93
Baseline ttP, min- median (IQR)	6.0 (5.3, 6.6)	6.0(5.3, 6.8)	0.96
Week 2 ttP, min- median (IQR)	6.3 (5.3, 7.2)	5.7 (5.3, 6.7)	0.22
Change in ttP, min-mean (SD)	0.4 (1.7)	-0.1 (1.2)	0.27
Baseline Peak, nmol-mean (SD)	36 ± 78)	327 ± 54	0.66
Week 2 Peak, nmol- mean (SD)	40 ± 80	338 ± 57	0.92
Change in Peak, nmol-mean (SD)	2 (73)	10 (39)	0.66
Other coagulation assays			
Baseline D-dimer , ng/mL-median (IQR)	1035 (540, 1685)	2280 (1590, 3940)	**0.001**
Week 2 D-dimer , ng/mL- median (IQR)	970 (460, 1900)	2240 (1280, 3940)	**0.001**
Change in D-dimer, ng/mL- mean (SD)	-130 (2150)	-408 (2726)	0.65
Baseline Fibrinogen, g/L - mean (SD)	3.9 ± 1.4	3.9 ± 1.4	0.85
Week 2 Fibrinogen, g/L- mean (SD)	4.6 ± 1.3	4.5 ± 1.4	0.73
Change in Fibrinogen, g/L	0.6 (1.3)	0.7 (1.4)	0.85

ETP- Endogenous thrombin potential, ttP-Time to peak, SD- Standard deviation, IQR- Interquatile range, DD-D-dimer

The effect of clinical and laboratory factors in order of utility in predicting DVT with corresponding binomial confidence intervals are shown in tables 3a-d. ROC curve for NIHSS (figure 3a), ROC curve for Barthel index (figure 3b), ROC curve for baseline D-dimer (figure 3c), ROC curve for week 2 D-dimer (figure 3d) are illustrated below.

D-dimer values at baseline and week 2 were better predictors of DVT with areas under the ROC curve (AUC) of 0.75 at both time points. Cut point at 50th centile for baseline (1660ng/mL) and week 2 D-dimer (1570ng/mL), gave 0.71 sensitivity (95% CI, 0.47-0.90) and 0.74 specificity (95% CI, 0.62-0.83) and 0.71 sensitivity (95% CI: 0.45-0.90), specificity 0.71(95% CI, 0.60-0.82) respectively. ROC curve analysis of Barthel index and NIHSS revealed they were the least predictors respectively, with AUC of 0.697 for Barthel index which gave 0.67 sensitivity (95% CI, 0.41-0.81) and 0.65 specificity (95% CI, 0.53-0.76) when the cut point is set at 6 (50th centile). The

AUC for NIHSS 50th centile cut-point of 14 was 0.633, which gave 0.64 sensitivity (95% CI, 0.41-0.87), and specificity of 0.53 (95% CI, 0.41-0.64).

Table 3, Prediction of Deep vein thrombosis at 50th centile cut-points using ROC curves.

Variables	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Baseline DD	1660ng/ml	0.71 (0.47, 0.90)	0.74 (0.62, 0.83)	0.39 (0.22, 0.58)	0.95 (0.83, 0.99)
Week 2 DD	1570ng/ml	0.71 (0.45, 0.90)	0.71 (0.60, 0.82)	0.38 (0.21, 0.56)	0.91 (0.80, 0.97)
Barthel Index	6	0.67 (0.41, 0.81)	0.65 (0.53, 0.76)	0.32 (0.18, 0.49)	0.89 (0.77, 0.96)
NIHSS	14	0.64 (0.41, 0.87)	0.53 (0.41, 0.64)	0.26 (0.14, 0.40)	0.87 (0.73, 0.95)

PPV, positive predictive value;. NPV, negative predictive value; NIHSS, national institute of health stroke scale; BI, barthel index; CI, confidence interval; DD, D-dimer

A multivariate logistic regression analysis was performed to jointly examine the association between factors tested and DVT, and a backward selection procedure was used to retain only statistically significant variable. Baseline D-dimer was the only significant variable after this analysis, with crude OR of 8.11 (95% CI: 1.90-34.7; p=0.005) for DVT as shown in table 4. Logarithm of baseline D-dimer was then undertaken, where a 10-fold increase in D-dimer which is equivalent to a 1-unit increase on the log scale is associated with odds of DVT, and this increased predictability by a factor of 8. In figure 4, is a graphical illustration showing a fitted relationship of the probability of DVT at different baseline D-dimer values.

Considering that Baseline D-dimer showed a multimodal distribution, dichotomisation was undertaken at median (1500ng/mL), 75th centile (4940ng/mL) and 90th centile (7600ng/mL) for the purpose of analysis. D-dimer showed a dose dependent trend towards association with DVT before adjustment for cofounders. D-dimer greater than 1500ng/ml was associated OR 4.10 (95% CI, 1.39-7.40; p=0.006) with increased odds of DVT. While D-dimer above the 75th and 90th percentile was associated with an OR 5.82 (95% CI, 1.72-8.40; p=0.003) and 6.91 (95% CI, 1.84-10.2; p=0.005) odds of DVT respectively. After adjustment for cofounders (age,

BMI, BI, NIHSS), D-dimer above the median showed significant association OR 2.95(95% CI 1.98-5.60; p=0.04). The adjusted OR for D-dimer above 75th and 90th centiles also remained significant at 3.89 (95% CI 2.10-9.80; p=0.002) and 3.4 (95% CI:1.17-11.4; p=0.003) respectively.

D-dimer measured in week 2 (DD2) was analysed as a continous variable after log transformation and after dichotomisation at the upperlimit of normal which was taken as 500ng/mL, revealed the unadjusted OR for log of DD2 4.32 (95% CI: 1.23- 14.3; p=0.003) for DVT. After adjustment for cofounders log of DD2 was 3.9 (95% CI 1.91-7.93; p=0.20). At week 2, a positive D-dimer was also associated with DVT with OR of 5.3 (95% CI 1.8-13.9; p=0.004). A summary of final model of the multivariable logistic regression analysis and predictors of DVT in unadjusted and adjusted analysis is table 4 below.

Table 4. Unadjusted and adjusted odds of association of predictors of DVT

Outcome	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value.
Age*	3.11(1.70-4.10)	0.02	1.89 (0.67-6.69)	0.20†
BMI**	4.90 (1.87-6.66)	0.05	3.20 (1.97-4.89)	0.06†
Barthel index***	5.1 (2.67-8.90)	0.08	4.2 (1.76- 8.40)	0.15†
NIHSS****	3.6 (1.03-9.11)	0.07	2.81 (0.73-8.33)	0.19†
DD1 ≥(Crude)	8.11 (1.90, 34.7)	0.005	-	
DD1 ≥1500ng/mL (median)	4.10 (1.39-7.40)	0.006	2.95 (1.98-5.60)	0.005†
DD1 ≥4940ng/mL (75th centile)	5.82 (1.72-8.40)	0.003	3.98 (2.10-9.80)	0.005†
DD1 ≥7600ng/mL (90th centile)	6.91 (1.84-10.2)	0.005	3.4 (1.17-11.4)	0.003†
Log DD2	4.32 (1.23-14.3)	0.03	3.9 (1.91-7.93)	0.20†
DD2 >500ng/mL	5.3 (1.80-13.9)	0.004	3.45 (0.76-9.40)	0.17†

(*) Analysed on the log scale (base 10) OR, odds ratio; CI, confidence interval; BMI, body mass index; DD1, D-dimer (baseline) time 1; DD2, D-dimer (week 2) time 2; NIHSS, national institute of health stroke scale; (*) per 10 year increase in age; (**) per 10mg/kg ^{2} increase in BMI;(***) per 4 unit decrease in Barthel index; (****) per 4 unit increase in NIHSS; (†) adjusted for age, NIHSS, BMI, barthel index; Log, logarithm

Discussion

In this study, the incidence of DVT remained high (19.6%) despite specialist care input and a significant proportion of patients receiving thrombolytic therapy. This contrasts with clinical experience on the association between good practices regarding specialist stroke care reduce incidence of DVT. Amongst participants receiving thrombolytic therapy, 21% had DVT and this constitute 40% of DVT in the ischaemic stroke group. The incidence of DVT in our patient population may be partially attributable to the existing guidelines in the United Kingdom, which do not support the use of pharmacological prophylaxis in acute stroke patients and theoretical risks of haemorrhagic transformation of infarct and probable extension of an intracerebral haematoma. Secondly, this incidence can also partly be explained by development of DVT prior to demonstration of neurological recovery and early mobilisation, considering most patients were mobilised 24 hours after thrombolytic therapy. Immobility due to motor limb weakness is a major risk factor for VTE (25-27), and development of thrombus in deep veins may have commenced as early as 48 hours following stroke (3, 4). In support of this we confirmed that DVT was associated with measures of impairment and disability after acute stroke. More patients with DVT had significant motor limb weakness, significant functional inability, with lower Barthel index, and greater stroke severity score with higher NIHSS. Thirdly, lack of protection against DVT in patients offered thrombolyic therapy may be due to short half life of r-tPA, it also suggest that early DVT prophylaxis in addition to the overall hyperacute stroke care is required. In previous studies, raised serum urea which can be a marker of dehydration, and other acute inflammatory markers, such as fibrinogen and CRP were associated with DVT (28-31). We did not demonstrate an association between DVT and fibrinogen.

Our study is the first to examine thrombin generation in the aftermath of acute stroke, and to demonstrate that D-dimer has potential clinical utility in suggesting at risk patients after acute stroke. We theorised that an increase in thrombin generation may indicate a predisposition to thrombus formation in the aftermath of acute stroke (32, 33), and therefore predict those patients that will eventually develop DVT. However, no association was found between thrombin generation and DVT. The lack of a link with thrombin generation, suggests that factors other than a generalised prothrombotic tendency determines the development of DVT after acute stroke. We

confirmed previous findings, that 2-point D-dimer values were significantly associated with DVT. D-dimer assay around day 9 has been shown to have good discriminatory power for predicting proximal DVT after acute ischaemic stroke (34). Harvey et al in a similar study (n=105) showed D-dimer assay within 24 hours of stroke, at cut point of 1591 ng/mL, gives 79% sensitivity, 78% specificity, 35% positive predictive value, and 96% negative predictive value (27). We found D-dimer assay within 48 hours of stroke at a cut-off of 1660 ng/mL had similar clinical utility in suggesting underlying DVT burden. At this cut point, 13/18 (72%) with asymptomatic DVT were identified. Baseline D-dimer is as effective as D-dimer measured at later time points in suggesting underlying risk of DVT, and as a result early elevation in D-dimers might be useful in risk stratifying patients need for thromboprophylaxis. D-dimer assay is a readily available, automated, and cheap test, in comparison to thrombin generation assay, and could therefore be easily integrated into a pretest assessment model even within the stroke population. Early initiation of pharmacological prophylaxis (within 48 hours) in studies of stroke patients showed significant reduction in VTE and with a non significant increased risk of intracerebral haemorrhage after acute ischaemic stroke (35, 36). Most international guidelines recommend pharmacological prophylaxis in immobile and high risk patients, but there is no clarity on the time of initiation (14-16, 36). Inclusion of D-dimer in future risk stratification criteria may assist in identifying those that will benefit from early detection. Early detection may further assist in making decisions on prompt initiation of thromboprophylaxis, and possibly limit the number needed to treat, and thereby reducing the overall risk of bleeding. Low Barthel index at a cut-off of ≤ 6 should raise clinical suspicion, but not as an independent factor to predict DVT risk as previously shown in a study were association with proximal DVT was found at a higher cut-off of ≤ 9 (37). Ogata et al, previously demonstrated an association between DVT and high NIHSS after acute haemorrhagic stroke (11) and at a threshold of ≥ 14, pharmacological prophylaxis was found to be safe and effective (36). At NIHSS of ≥ 14, 67% of DVT could have been predicted (67% sensitivity) but not as an independent factor. D-dimer remained the only independent factor with good clinical utility to predict DVT.

Limitations of this study relate to the population investigated, with 48.9% of those enrolled completing the follow up, this may not have altered the results on incidence

considering that those discharge early were ambulatory within 48 hours and are the groups less likely to have developed DVT and were more likely to return for follow up. Due to geographical location of the study centre as a regional hyperacute stroke referral centre, early repatriation was unavoidable, and follow up of participants with severe stroke in local hospitals was impractical due to differences in local hospital specialist input practices and differences in DVT imaging techniques. Adjustment was made for the expected missing data in our analysis. Application of our findings in participants with short hospital stay has limitations. Given the short duration of follow up, and that the risk of DVT remains up to 3 months(38, 39), it is possible a number of later DVT events were missed. Lastly, due to heterogenous patient population and small sample size, we are unable to undertake subgroup analysis in relation to stroke subtypes and those that received thrombolytic therapy.

In conclusion, we have demonstrated that DVT remains a common complication after acute stroke, despite thrombolytic therapy and improved specialist stroke care input, suggesting a need for additional thromboprophylaxis. In our study population D-dimer assay was independently associated with DVT, thus suggesting underlying DVT risk, when compared to other coagulation assays and measures of stroke severity. Further research to establish the role of D-dimer in identifying acute stroke patients at risk of DVT for early thromboprophylaxis is desirable.

Authors contributions

IO Balogun designed the study, collected, analysed data and drafted the manuscript. LN Roberts undertook laboratory investigations and critically reviewed the paper. R Patel and L Kalra designed the study and critically reviewed the manuscript. R Pathansali, R Arya designed the study, supervised the project and critically reviewed the paper. All authors agreed on final approval of the version to be published.

Acknowledgements

We are grateful to staff in the vascular laboratory under the supervision of Dr David Goss. We thank Paradzai Chitongo, Elizabeth Ford and biomedical scientists for performing D-dimer and Fibrinogen assays. We are equally grateful to participants, relatives, and multidisciplinary team members on Friend stroke unit at King's College Hospital, London for supporting recruitment into the study.

References:

1. Goodacre S, Sampson F, Stevenson M et al.. Measurement of the clinical and cost-effectiveness of non-invasive diagnostic testing strategies for deep vein thrombosis. Health technology assessment. 2006;10:1-168.

2. Spyropoulos AC, Anderson FA, Jr., Fitzgerald G et al. Predictive and associative models to identify hospitalized medical patients at risk for VTE. Chest. 2011;140:706-14.

3. Boeer A VE, Henze T, Prange HW et al. Early heparin therapy in patients with spontaneous intracerebral haemorrhage. Journal of neurology, neurosurgery, and psychiatry. 1991;54:466-7.

4. Turpie AG, Levine MN, Hirsh J et al. Double-blind randomised trial of Org 10172 low-molecular-weight heparinoid in prevention of deep-vein thrombosis in thrombotic stroke. Lancet. 1987;1:523-6.

5. Langhorne P. Measures to improve recovery in the acute phase of stroke. Cerebrovascular diseases. 1999;9:2-5.

6. Indredavik B, Bakke F, Solberg R et al. Benefit of a stroke unit: a randomized controlled trial. Stroke. 1991;22(8):1026-31.

7. Marler J. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. The New England journal of medicine. 1995;333:1581-7.

8. Skaf E, Stein PD, Beemath A et al. Venous thromboembolism in patients with ischemic and hemorrhagic stroke. The American journal of cardiology. 2005;96:1731-3.

9. Sun KK, Pang BS, Yang YH et al. The prevalence of deep venous thrombosis in hospitalized patients with stroke. Zhonghua yi xue za zhi. 2004;84:637-41.

10. HaraY. Deep venous thrombosis in stroke patients during rehabilitation phase. The Keio journal of medicine. 2008;57:196-204.

11. Ogata T, Yasaka M, Wakugawa Y et al. Deep venous thrombosis after acute intracerebral hemorrhage. Journal of the Neurological Sciences. 2008;272:83-6.

12. Warlow C OD, Douglas AS et al. Venous thrombosis following strokes. Lancet. 1972;1:1305-6.

13. Adams HP, del Zoppo G, Alberts MJ et al. Guidelines for the early management of adults with ischemic stroke. Circulation. 2007;115:478-534.

14. Lindsay P, Bayley M, McDonald A et al. Toward a more effective approach to stroke: Canadian Best Practice Recommendations for Stroke Care. Canadian Medical Association Journal. 2008;178:1418-25.

15. Hacke W. Guidelines for Management of Ischaemic Stroke and Transient Ischaemic Attack 2008. Cerebrovascular Diseases. 2008;25:457-507.

16. Shinohara Y. Outline of the Japanese Guidelines for the Management of Stroke 2004 and subsequent revision. International journal of stroke. 2008;3:55-62.

17. Tripodi A, Legnani C, Chantarangkul V et al. High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of

recurrent venous thromboembolism. Journal of thrombosis and haemostasis. 2008;6:1327-33.

18. Tripodi A, Legnani C, Palareti G et al. More on: high thrombin generation and the risk of recurrent venous thromboembolism. Journal of thrombosis and haemostasis. 2009;7:906-7.

19. van Hylckama Vlieg A, Christiansen SC, Luddington R et al. Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence. British journal of haematology. 2007;138:769-74.

20. Dargaud Y TM, Bordet JC, Ninet J et al. Use of calibrated automated thrombinography +/- thrombomodulin to recognise the prothrombotic phenotype. Thrombosis and haemostasis. 2006;96:562-7.

21. ten Cate-Hoek AJ, Dielis AW, Spronk HM et al. Thrombin generation in patients after acute deep-vein thrombosis. Thrombosis and haemostasis. 2008;100:240-5.

22. Roberts LN PR, Bonner L, Arya R et al. African-Caribbean ethnicity is associated with a hypercoagulable state as measured by thrombin generation. Blood coagulation & fibrinolysis. 2013;24:40-9.

23. Hemker HC, Giesen P, Al Dieri R et al. Calibrated Automated Thrombin Generation Measurement in Clotting Plasma. Pathophysiology of haemostasis and thrombosis. 2003;33(1):4-15.

24. Bagot CN, Marsh MS, Whitehead M et al. The effect of estrone on thrombin generation may explain the different thrombotic risk between oral and transdermal hormone replacement therapy. Journal of thrombosis and haemostasis. 2010;8:1736-44.

25. Harvey RL. Prevention of venous thromboembolism after stroke. Topics in stroke rehabilitation. 2003;10:61-9.

26. Landi G, D'Angelo A, Boccardi E, Candelise L et al. Venous thromboembolism in acute stroke. Prognostic importance of hypercoagulability. Archives of neurology. 1992;49:279-83.

27. Harvey RL RE, Yarnold PR, Durham JR et al. Deep vein thrombosis in stroke. The use of plasma D-dimer level as a screening test in the rehabilitation setting. Stroke. 1996;27:1516-20.

28. Kelly J HB, Lewis RR, Swaminathan R et al. Dehydration and venous thromboembolism after acute stroke. QJM. 2004b;97:293-6.

29. Reiter M BR, Koca N, Dirisamer A et al. Deep vein thrombosis and systemic inflammatory response: a pilot trial. Wiener klinische Wochenschrift. 2003;115:111-4.

30. Bucek RA, Quenhenberger P, Minar E et al. C-reactive protein in the diagnosis of deep vein thrombosis. British journal of haematology. 2002;119:385-9.

31. Roumen-Klappe EM, den Heijer M, van Uum SH et al. Inflammatory response in the acute phase of deep vein thrombosis. Journal of vascular surgery. 2002;35:701-6.

32. Gast A TT, Baumgartner HR. Thrombin plays a key role in late platelet thrombus growth and/or stability. Arteriosclerosis and thrombosis. 1994;14:1466-74.

33. Borissoff JI, Spronk HMH, Heeneman S et al. Is thrombin a key player in the ‘coagulation-atherogenesis’ maze? Cardiovascular research. 2009;82:392-403.

34. Kelly J RA, Lewis RR, Parmar K et al. The relationship between acute ischaemic stroke and plasma D-dimer levels in patients developing neither venous thromboembolism nor major intercurrent illness. Blood coagulation & fibrinolysis. 2003a;14:639-45.

35. Kamphuisen PW. What is the optimal pharmacological prophylaxis for the prevention of deep-vein thrombosis and pulmonary embolism in patients with acute ischemic stroke? Thrombosis research. 2007;119:265-74.

36. Sherman DG, Albers GW, Bladin C et al. The efficacy and safety of enoxaparin versus unfractionated heparin for the prevention of venous thromboembolism after acute ischaemic stroke (PREVAIL Study): an open-label randomised comparison. Lancet. 2007;369:1347-55.

37. Kelly J RA, Lewis RR, Coshall C et al. Screening for proximal deep vein thrombosis after acute ischemic stroke: a prospective study using clinical factors and plasma d-dimers. Journal of Thrombosis and Haemostasis. 2004a;2:1321-6.

38. Kearon C. Natural History of Venous Thromboembolism. Circulation. 2003;107:22-30.

39. Kearon C. Natural history of venous thromboembolism. Seminars in vascular medicine. 2001;1:27-37.

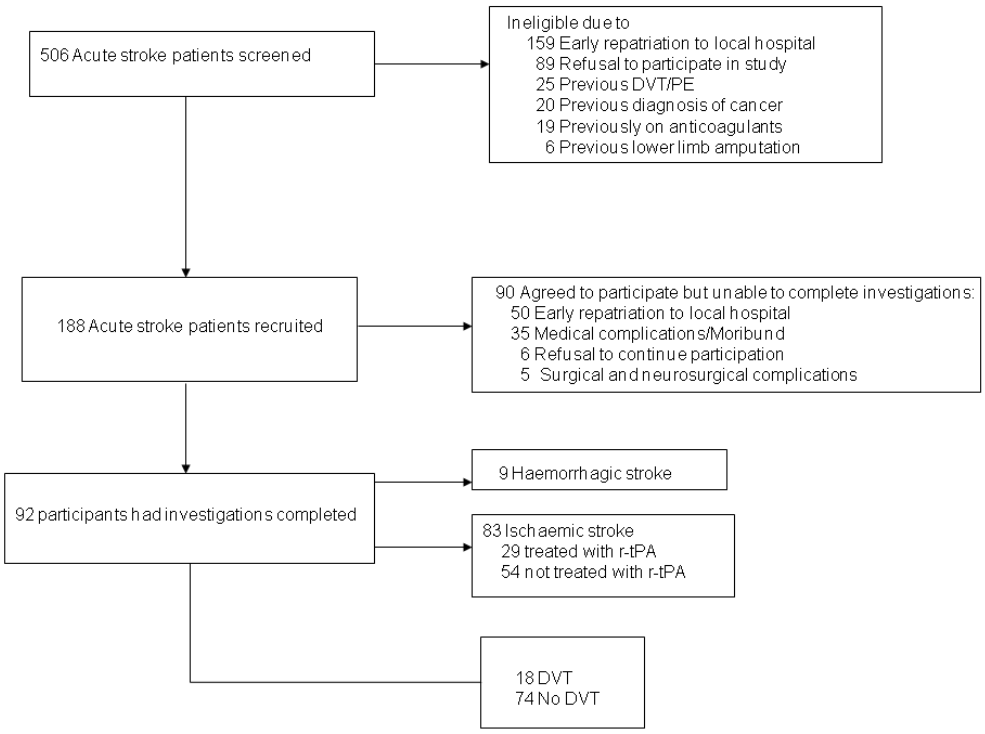

Fig. 1

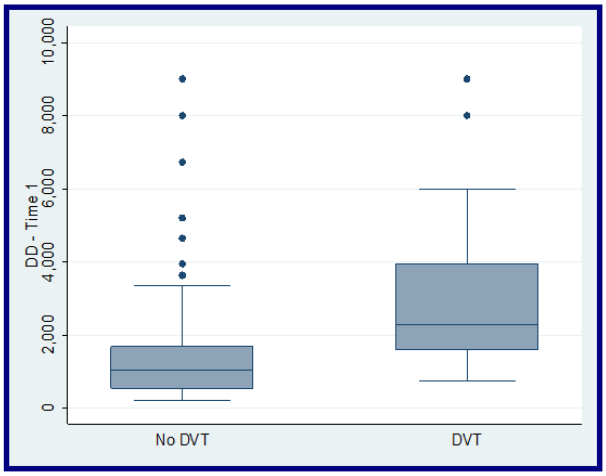

Fig. 2a

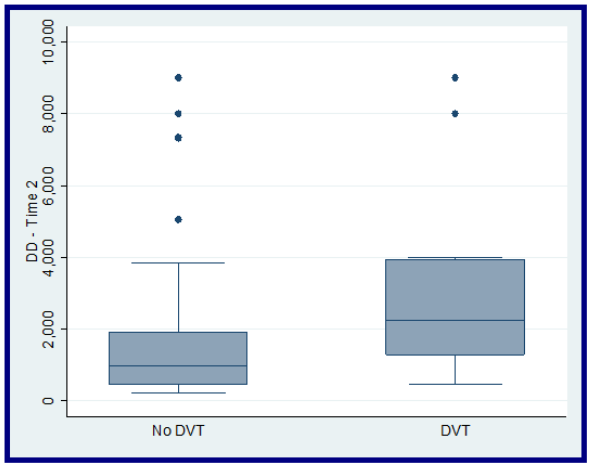

Fig. 2b

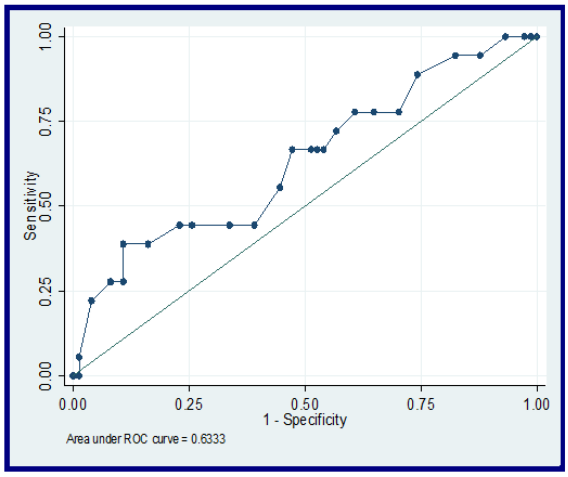

Fig. 3a

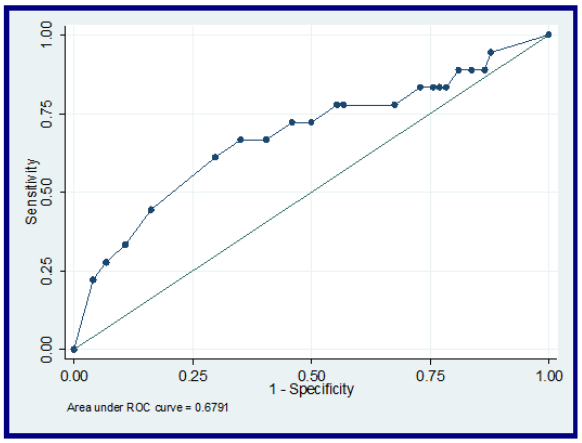

Fig. 3b

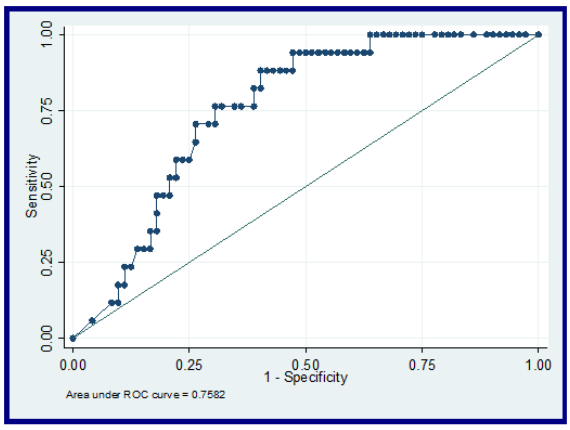

Fig. 3c

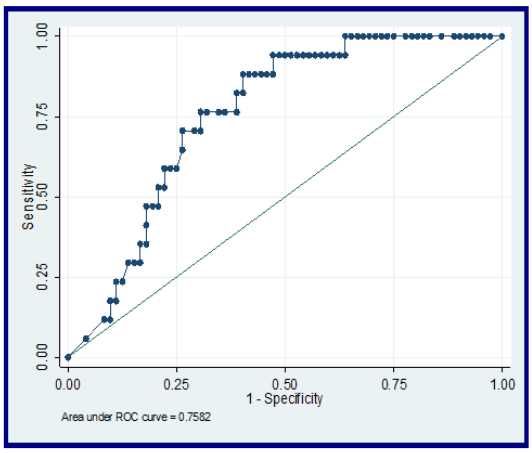

Fig. 3d

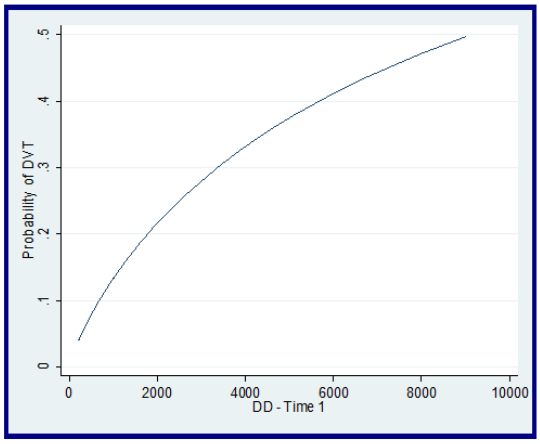

Fig. 4

Highlights

Clinical and laboratory predictors of DVT (Highlight of key findings)

What we knew

- Subclinical deep vein thrombosis remain a common clinical problem after acute stroke
- Baseline D-dimer within 48 hours of admission best predict post stroke DVT
- Baseline D-dimer ≥1500ng/mL has >70% sensitivity and specificity in the prediction of post stroke DVT
- Inclusion of admission D-dimer in DVT prediction model is desirable for future investigations

What is new

- Thrombin generation has no clinical utility in the prediction of post stroke DVT
- Thrombolytic therapy does not protect against post stroke DVT